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# Metabolism, tissue disposition, and excretion of 1,2-*bis*(2,4,6-tribromophenoxy)ethane (BTBPE) in male Sprague-Dawley rats

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**Abstract**

A single oral dose of [$^{14}$C] 1,2-*bis*(2,4,6-tribromophenoxy)ethane (BTBPE) was administered to conventional and bile-duct cannulated male Sprague-Dawley rats. Tissue disposition, excretion and metabolism was determined. BTBPE is a low-volume brominated flame retardant used in resins or plastics, and toxicity data in peer-reviewed journals is extremely limited. BTBPE was fairly insoluble in lipophilic solutions, which made dose preparation difficult. The great majority of $^{14}$C (>94%) was excreted in the feces of both groups of rats at 72 h, and tissue retention was minimal. Lipophilic tissues contained the highest concentrations of BTBPE, e.g. thymus, adipose tissue, adrenals, lung, and skin. Metabolites were excreted in the urine, bile and feces, but at a very low level. Fecal metabolites were characterized as monohydroxylated, monohydroxylated with debromination, dihydroxylated/debrominated on a single aromatic ring, monohydroxylated on each aromatic ring with accompanying debromination, and cleavage on either side of the ether linkage to yield tribromophenol and tribromophenoxyethanol. Despite a limited quantity of stable metabolites extractable in the feces, non-extractable $^{14}$C levels were relatively high (39% of the 0–24 h fecal $^{14}$C), which suggested that BTBPE could be metabolically activated in the rat and covalently bound to fecal proteins and/or lipids. It was concluded that limited absorption and metabolism of BTBPE would occur by ingestion in mammals.


*Keywords:* *Bis*(2,4,6-tribromophenoxy)ethane (BTBPE); Brominated flame retardants; Pharmacokinetics; Metabolism; Mammal; Mass spectrometry

## 1. Introduction

1,2-*Bis*(2,4,6-tribromophenoxy)ethane (BTBPE) is a brominated flame retardant (BFR) used in the production of plastic materials that require high manufacturing temperatures and light stability, for acrylonitrile-butadiene-polystyrene, and high impact polystyrene. Production figures are difficult to obtain, but current US production exceeds 450 Mt (Environmental Defense, 2002), and according to the World Health Organization (WHO, 1997) BTBPE is classified as a low-volume BFR. BTBPE is hydrophobic ($K_{ow} = 3.14$), and, like many BFRs, would be expected to be persistent in the environment. However, it is possible that ether cleavage of BTBPE may yield 2,4,6-tribromophenol, which has been reported to be a developmental neurotoxicant (Lyubimov et al., 1998). Environmental levels of BTBPE have not been reported to our knowledge, although an occupational study revealed that humans are exposed to BTBPE in certain workplace environments (Sjödin et al., 2001).

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The purpose of the present study was to administer a single oral dose of BTBPE to male rats, and measure the tissue distribution and excretion behavior of BTBPE. Metabolites in the feces were characterized by chromatographic and spectral analyses.

## 2. Materials and methods

### 2.1. Chemical synthesis

[$^{12}$C] and [$^{14}$C] BTBPE were synthesized in-house by adding bromine water to uniformly labeled [UL-$^{14}$C] phenol (1.05 mCi/mmol; Sigma Chemical, St. Louis, MO) which yielded 2,4,6-tribromophenol quantitatively. Tribromophenol was refluxed overnight with ethylene dibromide in 20 ml acetone and 4 ml dimethylformamide using $K_2CO_3$ (0.66 g) as a catalyst according to the method of Kohn and Fink (1923). The purity of radiolabeled BTBPE (>98%) was verified by silica gel thin layer chromatography (TLC) (mobile phase 1:1 hexane:benzene) and reversed phased HPLC (80–100% methanol in water gradient over 20 min; $8 \times 100$ mm, C-18 DeltaPak, Waters Associates, Milford, MA). The chemical purity of the unlabeled BTBPE (>95%) was verified by $^1$H-NMR and gas chromatography/mass spectrometry (GC/MS) in electron impact mode.

### 2.2. Dose preparation

The radiolabeled BTBPE was diluted with unlabeled chemical until the desired specific activity was achieved (1355 dpm/μg). BTBPE solubility was low in all the common vehicles used in oral dose preparations. Peanut oil was used for dosing, but was warmed to keep BTBPE in solution prior to dosing. Toluene and anisole are the best solvents for BTBPE, but were toxic, and could not be diluted with peanut oil without precipitation. Lutrol F127: soya phospholipid (34:16 w:w; BASF Corporation and Nattermann Phospholipid GmbH) diluted with water was also unsuccessful as a vehicle, because addition of water caused an immediate precipitation from solution.

### 2.3. Animals

The radiolabeled BTBPE (2.0 mg/kg in 0.5 ml peanut oil; 1.23 μCi/rat) was administered orally via a stomach tube device constructed of a 1.0-ml glass syringe fitted with 15 cm of Teflon tubing (1/32″) to seven conventional and six bile-duct cannulated male rats (283–328 g; Sprague-Dawley, Taconic Labs, Germantown, NY, USA). Both groups of rats were housed in a controlled environment of 20 °C, 12 h light/dark cycle, and a relative humidity of 25% ± 5%. The cannulated rats were anaesthetized with halothane prior to surgery. A ventral midline incision was made, and the bile duct was cannulated with medical polyethylene tubing 1.5 cm from the base of the liver. The cannula was fastened with 4-0 silk ligature, and exited near the base of the tail. The rats were housed in stainless steel metabolism cages, and were allowed free access to water and feed (Lab Diet 5012, PMI Nutritional International, Brentwood, MO). Urine, feces, and bile were collected at 24-h intervals for 72 h. The rats were anesthetized with halothane and killed by exsanguination. Adrenals, epididymal fat, G.I. tract, heart, kidneys, liver, lungs, spleen, testes, and thymus were removed.

### 2.4. Tissue and excreta analyses

Urine, bile, and blood were assayed for radioactivity by counting aliquots in a liquid scintillation counter (LSC). Lyophilized feces and tissues were combusted in a tissue oxidizer, and the $^{14}$C counted by LSC. The pooled, lyophilized feces were extracted sequentially with anisole, acetone, methanol, and water (500 ml, 24 h, three times with stirring). The fecal residue was then extracted further with an accelerated solvent extractor (ASE-200, Dionex Corporation, Sunnyvale, CA, USA) at 1500 psi and 100 °C using hexane, ethyl acetate and methanol as solvents (30 min each). Reversed phase C-18 HPLC analyses of fecal extracts were performed using a linear gradient of 20% water/methanol to 100% methanol. HPLC fractions were evaporated to dryness on a rotary evaporator and submitted for GC/MS analysis. The metabolites were characterized by GC/MS in electron impact mode after derivatization to either the trimethylsilyl (TMS) or methyl ether with Regisil® (Regis Technologies, Inc., Morton Grove, IL, USA) or diazomethane (prepared in-house), respectively. GC/MS was performed on a VG Autospec mass spectrometer operating at 70 eV, using a Model 5890 GC (Hewlett–Packard, Palo Alto, CA) with a 15-m DB-5ms column (J&W Scientific, Folsom, CA) run from 70 to 310 °C at 10 °C/min. Spectra by $^1$H-NMR were obtained on a Bruker AM-400 spectrometer (Billerica, MA), and referenced to tetramethylsilane ($\delta$ 0.00 ppm). Protein in urine and bile was removed by methanol precipitation. The supernatants were acidified with 1 M HCl, then applied separately to solid phase extraction columns (ENVI-18, Supelco, Bellafonte, PA, USA), eluted sequentially with water and methanol, and the eluants were applied to a silica gel TLC plate (Analtech, Inc., Newark, DE, USA). Mobile phases for TLC development were (a) 1:1 hexane:benzene, which distinguished between parent and metabolites, (b) 1:1:2 tetrahydrofuran:ethyl acetate:hexane, which resulted in the migration of Phase I metabolites off the origin, and (c) 27:27:4 toluene:methanol:glacial acetic acid, which resulted in the migration of acidic conjugates from the origin.

## 3. Results

### 3.1. Excretion

In the urine of conventional rats, cumulative excretion of $^{14}C$ at 72 h was only 1.6% of the dose, and slightly more than 0.03% in the bile-duct cannulated rats (Table 1). Fecal excretion was high at 24 h for conventional and bile-duct cannulated rats, i.e. 93% and 58%, respectively, and declined rapidly with time. Cumulative fecal excretion was greater than 100% for conventional rats, probably due to dose variability arising from solubility problems, and was 94% for bile-duct cannulated rats.

### 3.2. Tissue disposition

As a consequence of the high fecal excretion, low tissue levels of BTBPE were observed. Only the residual carcass and GI tract contained greater than 0.1% of the dose at 72 h in conventional rats (Table 2). The residual carcass consisted of skin, abdominal fat, muscle, and bone. Assuming 55 g of skin/rat, almost 0.9% of the dose was in the skin, and the remainder was assumed to be in the lipophilic abdominal fat. Since the GI tract was not flushed of its contents, it was likely that the majority of $^{14}C$ was due to residual feces. Approximately 0.03% of the BTBPE dose was detected in the liver and epididymal fat of conventional rats. On a concentration basis, the more lipophilic tissues contained the highest concentration of $^{14}C$. If it is assumed that tissue $^{14}C$ was parent BTBPE, then the GI tract, thymus, adipose tissue, adrenals and lungs, contained the highest concentration of BTBPE, i.e. >0.5 nmol/g tissue (Table 2). Skin, liver, and carcass had intermediate concentrations, 0.2–0.5 nmol/g tissue, and kidney, spleen, heart, testes and blood had low concentrations, i.e. <0.1 nmol/g tissue.

Table 1
Recoveries of $^{14}C$ from male rats dosed orally with [$^{14}C$] 1,2-*bis*(2,4,6-tribromophenoxy)ethane (BTBPE) in conventional and bile-duct cannulated experiments

| Excreta | Percent of dose | |
|---|---|---|
| | Conventional ($n = 7$) | Cannulated ($n = 6$) |
| *Urine* | | |
| 0–24 h | 1.1 ± 0.22 | 0.01 ± 0.009 |
| 24–48 h | 0.4 ± 0.11 | 0.005 ± 0.011 |
| 48–72 h | 0.09 ± 0.05 | 0.02 ± 0.031 |
| *Bile* | | |
| 0–24 h | – | 0.2 ± 0.11 |
| 24–48 h | – | 0.02 ± 0.02 |
| 48–72 h | – | <0.01 |
| *Feces* | | |
| 0–24 h | 92.8 ± 16.2 | 58.1 ± 33.6 |
| 24–48 h | 20.5 ± 6.7 | 30.8 ± 26.8 |
| 48–72 h | 0.7 ± 0.2 | 5.8 ± 6.8 |
| Total | 115.6 | 95.0 |

### 3.3. Metabolite characterization

Approximately 39% of the $^{14}C$ from 0 to 24 h feces in conventional rats was non-extractable with liquid extraction using anisole, acetone, methanol and water. This figure increased to 44% with 24–48 h conventional rat feces and 83% with 48–72 h feces. To ascertain whether incomplete extraction with liquid extraction had occurred, or whether covalent bonding of formed BTBPE metabolites was responsible for the high non-extractable amounts of $^{14}C$, the fecal residues were further extracted with high pressure and temperature using an accelerated solvent extractor. The additional amounts of $^{14}C$ extracted were 1.6%, 1.6% and 0% from 0 to 24, 24 to 48, and 48 to 72 h feces, respectively.

The majority (>82%) of the extractable $^{14}C$ in 0–24 h feces was parent BTBPE [GC/MS: $M^{+}$ 682 (6 Br), M-327 (355, 3 Br), M-355 (327, 3 Br), M-383 (299, 3 Br); 1H-NMR: 7.65 (s) and 4.41 (s)]. Seven different, unconjugated metabolite structures in 0–24 h feces extracts were observed, which accounted for 2.7% of the administered dose. No stereochemical assignments or quantitation of individual metabolites were possible due to lack of authentic standards. The mass spectral results demonstrated that metabolism of BTBPE fell into two general categories. The first category of metabolites arose from multiple oxidations and debrominations of aromatic rings. The second category of metabolites were formed by cleavage on either side of the ether linkage resulting in monoaromatic ring metabolites.

Monohydroxylation of BTBPE was evident in the methylated mass spectrum of metabolite I (Table 3), where a six bromine isotope cluster at $M^{+}$ 712 was observed. Loss of 357 mass units to the three bromine ion at $m/z$ 355 corresponded to the loss of a methoxy-tribromophenolate ion. The loss of 355 mass units to a three bromine ion at $m/z$ 357 demonstrated the complementary nature of these two fragment ions. Monohydroxylation was also accompanied by debromination. Metabolite II was present as two isomers when methylated (Table 3) with different retention times on GC, each with a molecular ion of 634 (5 Br). A loss of 279 to $m/z$ 355 was compatible with a loss of a methoxy-dibromophenolate ion, and the complementary ion at $m/z$ 279 was also evident. Corroboration of this assignment was provided by the TMS derivative which had a molecular ion of 692 and a methyl fragment loss at 677 (Table 3).

Dihydroxylation combined with debromination of BTBPE was evident in the mass spectrum of methylated

Table 2
Tissue recoveries and concentrations of $^{14}C$ from male rats dosed orally with [$^{14}C$] 1,2-*bis*(2,4,6-tribromophenoxy)ethane (BTBPE) in peanut oil (2.0 mg/kg body weight) in conventional and bile-duct cannulated experiments

| Tissue | Percent of dose | | |
|---|---|---|---|
| | Conventional ($n = 7$) | Concentration | Cannulated ($n = 6$)[a] |
| Adrenals | $0.002 \pm 0.0005$ | 1.0 | $0.0002 \pm 0.003$ |
| Adipose (epid.) | $0.01 \pm 0.004$ | 1.2 | $0.0001 \pm 0.00009$ |
| Blood plasma | 0 | 0 | 0 |
| Carcass | $1.5 \pm 0.2$ | 0.2 | $0.6 \pm 0.6$ |
| Skin[b] | $0.9 \pm 0.4$ | 0.4 | $0.07 \pm 0.1$ |
| G.I. tract | $0.4 \pm 0.07$ | 1.5 | $1.5 \pm 2.3$ |
| Heart | $0.03 \pm 0.05$ | 0.08 | $0.0001 \pm 0.002$ |
| Kidney | $0.008 \pm 0.002$ | 0.1 | $0.0005 \pm 0.0007$ |
| Liver | $0.03 \pm 0.003$ | 0.2 | $0.06 \pm 0.1$ |
| Lungs | $0.009 \pm 0.005$ | 0.8 | $0.0006 \pm 0.0007$ |
| Spleen | $0.003 \pm 0.003$ | 0.1 | $0.0003 \pm 0.0007$ |
| Testes | $0.003 \pm 0.002$ | 0.02 | $0.0001 \pm 0.0002$ |
| Thymus | $0.02 \pm 0.02$ | 1.3 | $0.00001 \pm 0.00003$ |
| Total | 2.0 | | 2.2 |

The average concentration in conventional rat tissues was calculated by assuming all tissue radioactivity was parent (MW 682), and is expressed as nmol BTBPE/g tissue on a fresh weight basis.

[a] Tissue concentrations for cannulated rats were determined, but were very low, and therefore, not reported in the table.

[b] Percent of dose was estimated by taking concentration and multiplying by an estimated 55 g skin/rat.

metabolite III, which existed as two isomers. One of the isomers contained both hydroxyls on the same ring, since a five bromine molecular ion of the methylated derivative was evident at $M^+$ 664. A fragment loss of a tribromophenolate ion to $m/z$ 353 indicated the presence of a non-oxidized ring (M-311; Table 3). In addition, a fragment loss of 355 to $m/z$ 309 is compatible with a loss of a dimethoxy-dibromophenolate ion from the molecular ion, where both methoxys were on the same ring. The other isomer of methylated metabolite III existed with one hydroxyl group in each ring, and was revealed by a molecular ion loss of a monomethoxy-tribromophenolate fragment ion at $m/z$ 307, and a loss of a monomethoxy-dibromophenolate fragment ion at $m/z$ 385 (Table 3). The three bromine ion cluster for monomethoxy-tribromophenolate and the two bromine isotope cluster for monomethoxy-dibromophenolate fragment ions were also evident ($m/z$ 357 and 279, respectively; Table 3). Metabolite IV was characterized by MS following methylation as a dihydroxyl-*bis*(dibromophenoxy)ethane ($M^+$ 586; 4 Br). Each hydroxyl group was on a different aromatic ring. Fragment ion losses of 279 and 307 were losses of methoxy-dibromophenolate and methoxy-(dibromophenoxy)ethyl ions, respectively ($m/z$ 307 and 279; Table 3). In addition, a putative methoxy-dibromofuran rearrangement ion was evident at $m/z$ 251 (2 Br).

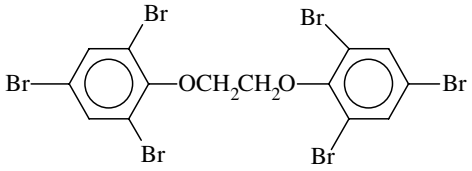
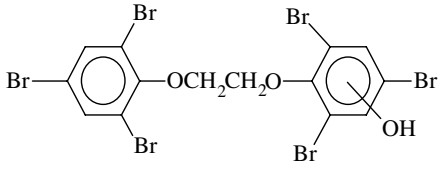
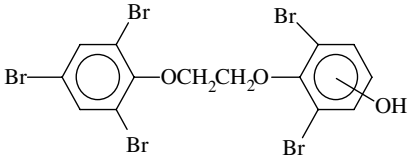
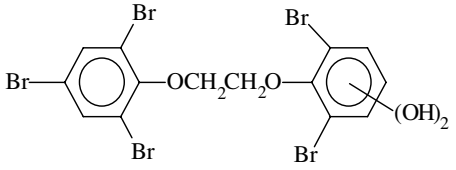
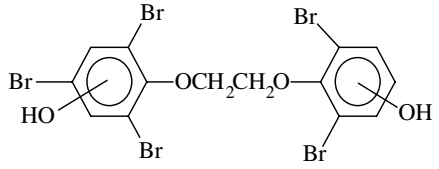
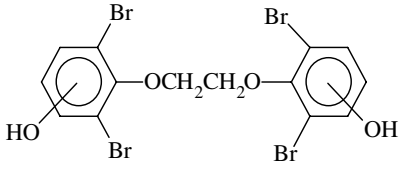
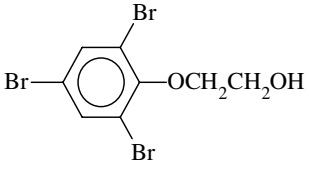
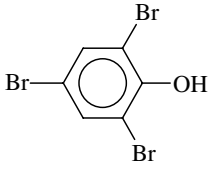
Metabolic cleavage on either side of the ether linkages was also observed in the formation of 2,4,6-tribromophenoxyethanol (metabolite V) and 2,4,6-tribromophenol (metabolite VI; Table 3). The TMS mass spectrum of metabolite V displayed a molecular ion at 444 (3 Br) and a diagnostic methyl loss to $m/z$ 429 (Table 3), which is consistent with the TMS ether of 2,4,6-tribromophenoxyethanol. The underivatized molecular ion was also evident as a three bromine ion cluster at $M^+$ 372. Silylation of metabolite VI with Regisil® produced a molecular ion at 400 (3 Br) and a methyl fragment ion loss at $m/z$ 385 (Table 3). Both fragment ions are consistent with a structure of 2,4,6-tribromophenol. In addition, the underivatized molecular ion was apparent at $M^+$ 328.

Analysis of urine and bile by TLC mobile phase (a) (see Section 2) showed only trace amounts of parent compound (data not shown). In the bile, both conjugated and unconjugated metabolites were suggested by utilizing TLC solvent systems (b) and (c) on silica gel plates (data not shown).

## 4. Discussion

It would initially appear that intestinal absorption of BTBPE was very poor since observed cumulative fecal excretions were greater than 100% and 94% for conventional and bile-duct cannulated rats, respectively, and approximately 82% of the extractable fecal $^{14}C$ was parent BTBPE. In addition, since cumulative biliary excretion of BTBPE was only 0.22% of the dose, hepatic metabolism of BTBPE would appear to be very low (Table 1). Previous work concluded that BTBPE was very poorly absorbed from the gut when administered

Table 3
Mass spectral data for BTBPE metabolites isolated from rat feces

| | |
|---|---|
|  | *Parent BTBPE*<br>$M^+$ (682, 6 Br); M-327 (355, 3 Br); M-355 (327, 3 Br); M-371 (311, 3 Br); M-383 (299, 3 Br) [EI-GC/MS of parent] [$^1$H-NMR]: 7.65 (s); 4.41 (s) |
|  | *Metabolite I*<br>$M^+$ (712, 6 Br); M-327 (385, 3 Br); M-355 (357, 3 Br); M-357 (355, 3 Br); M-385 (327, 3 Br) [as methyl ether] |
|  | *Metabolite II*<br>$M^+$ (634, 5 Br); M-279 (355, 3 Br); M-327 (307, 2 Br); M-355 (279, 2 Br) [as methyl ether]<br>$M^+$ (692, 5 Br); M-15 (677, 5 Br) [as trimethylsilyl ether] |
| 2 Isomers | |
|  | *Metabolite III—Isomer 1*<br>$M^+$ (664, 5 Br); M-311 (353, 2 Br); M-309 (355, 2 Br) [as methyl ether] |
|  | *Metabolite III—Isomer 2*<br>$M^+$ (664, 5 Br); M-279 (385, 3 Br); M-307 (357, 3 Br); M-357 (307, 2 Br); M-385 (279, 2 Br) [as methyl ether] |
|  | *Metabolite IV*<br>$M^+$ (586, 4 Br); M-279 (307, 2 Br); M-307 (279, 2 Br); M-335 (251, 2 Br) [as methyl ether] |
|  | *Metabolite V*<br>$M^+$ (444, 3 Br); M-15 (429, 3 Br) [as trimethylsilyl ether]<br>$M^+$ 372 (3 Br; underivatized metabolite) |
|  | *Metabolite VI*<br>$M^+$ (400, 3 Br); M-15 (385, 3 Br) [as trimethylsilyl ether]<br>$M^+$ 328 (3 Br; underivatized metabolite) |

Metabolites were characterized as their TMS ethers with Regisil®, or methyl ethers with diazomethane.

for one day at 0.05%, 0.5%, and 5% of the diet (Nomeir et al., 1993). Less than 1% was eliminated in the urine, while greater than 99% was excreted in the feces as parent. However, in the present study, 39% of the 0–24 h fecal $^{14}C$ from conventional rats was not extractable into solvents ranging from anisole to water. This value increased to 44% and 83% in 24–48 h and 48–72 h feces, respectively. Only an additional 1.6%, 1.6% and 0%, respectively, of the $^{14}C$ could be extracted using accelerated solvent extraction. This provided circumstantial evidence that BTBPE was metabolically activated and subsequently covalently bound to fecal proteins and/or lipids (Ansari et al., 1995; Dodds, 1995; Örn and Klasson-Wehler, 1998).

Three possible mechanisms may explain the observed results of high non-extractable fecal radioactivity. First, lumenomucosal circulation of BTBPE may have occurred by intestinal absorption and metabolic activation by the intestinal epithelial cells, followed by direct excretion into the gut. This mechanism has been shown to occur for propachlor (Struble, 1991), but apparently requires xenobiotics which already have activated functional groups in order for Phase II conjugates to form, which are then excreted into the gut. Since BTBPE would have required biological activation, e.g. cytochrome P450-mediated oxidation, lumenomucosal circulation is an unlikely explanation for the non-extractable $^{14}C$. Secondly, BTBPE may have undergone bioactivation by the gut microflora, a process which is essential for enterohepatic circulation, but which would increase the residence time of BTBPE in the body (Renwick, 1986). Enterohepatic circulation was not suggested by the excretion results, since elimination of BTBPE in urine, bile and feces peaked at 0–24 h in all cases, and virtually no $^{14}C$ was excreted in the bile (Table 1). Furthermore, the most common reactions by the gut microflora are hydrolyses and reductions, rather than oxidations and conjugations (Scheline, 1973; Renwick, 1976). The third, and most likely, possibility is that the absorbed BTBPE arrived at the liver from the portal vein, and was activated by hepatic cytochrome P450 enzymes into stable metabolites and reactive intermediates. The reactive intermediates could have been directly excreted in the bile, where spontaneous covalent bond formation with fecal proteins and/or lipids would have produced the non-extractable residues.

The biotransformation of BTBPE, as determined by the metabolites detected in the feces, included a series of oxidations, debrominations, and ether cleavages. The oxidation of aromatic compounds is usually mediated by cytochrome P450s, which initially produce an arene oxide (Fig. 1). Enzymatic or non-enzymatic ring opening of the arene oxide of BTBPE would have lead to the formation of a monohydroxylated compound (metabolite I). Often this ring opening is accompanied by an NIH shift of the halogen atom. However, no hydroxylated synthetic standards were available, and in their absence it was not possible to determine which position was oxidized or whether an NIH shift had occurred. Concomitant oxidation and dehalogenation of the arene oxide may have produced metabolite II. Alternatively, epoxide hydrolase and dihydrodiol dehydrogenase action on the arene oxide may have lead to the formation of metabolite III, isomer 1, which was a dihydroxy-pentabromo metabolite of BTBPE (Fig. 1).

Both aromatic rings of BTBPE were apparently subject to cytochrome P450 oxidation, either in series or simultaneously. Ring opening and dehalogenation of both arene oxides could have produced metabolite IV, which was dihydroxyl-*bis*(dibromophenoxy)ethane, while dehalogenation at only one of the two putative arene oxides would have produced metabolite III, isomer 2 (Fig. 1). Metabolite III, isomer 2 was distinguished from isomer 1 by MS, where it was clear from fragment ions that isomer 1 contained both hydroxyls on one aromatic ring, while on isomer 2 the hydroxyls were on different rings (Table 3).

Finally, BTBPE also underwent cleavage on either side of the ether linkage (Fig. 1). Metabolism on the aromatic side of the ether oxygen resulted in the formation of 2,4,6-tribromophenoxyethanol (metabolite V), which was consistent with not only an underivatized molecular ion at 372, but also a TMS-ether molecular ion and M-15 fragment ion at 444 and 429, respectively (Table 3). Similarly, in vivo cleavage on the aliphatic side of the ether oxygen lead to 2,4,6-tribromophenol ($M^{+\cdot}$ 400; metabolite VI; Table 3), although bromine atom rearrangements may have occurred, and in the absence of standards could not be determined with absolute certainty.

Cumulative tissue retention of BTBPE in conventional rats was only 2% of the administered dose at 72 h (Table 2). Most of the tissue $^{14}C$ was in the residual carcass (1.5%), which contained abdominal fat, skin, muscle and bone. Lipophilic tissues contained the highest concentrations of BTBPE, e.g. thymus, adipose tissue, adrenals, lung, and skin (Table 2). The liver to fat ratio in conventional rats was only 0.15. This indicated that BTBPE did not serve as a ligand for hepatic proteins, and that disposition was largely based on tissue lipophilicity. Toxic polychlorinated dioxins and furans have been shown to be both inducers and ligands for the hepatic cytochrome P450 1A2 (Diliberto et al., 1999) and have liver to fat ratios in excess of unity (Van den Berg et al., 1994).

In agreement with these results, adipose tissue, skin and thymus were the only tissues with measurable radioactivity following a single feeding of BTBPE in a previous study (Nomeir et al., 1993). However, following 10 consecutive days of feeding with spiked feed, all tissues contained $^{14}C$. The tissues with the highest con-

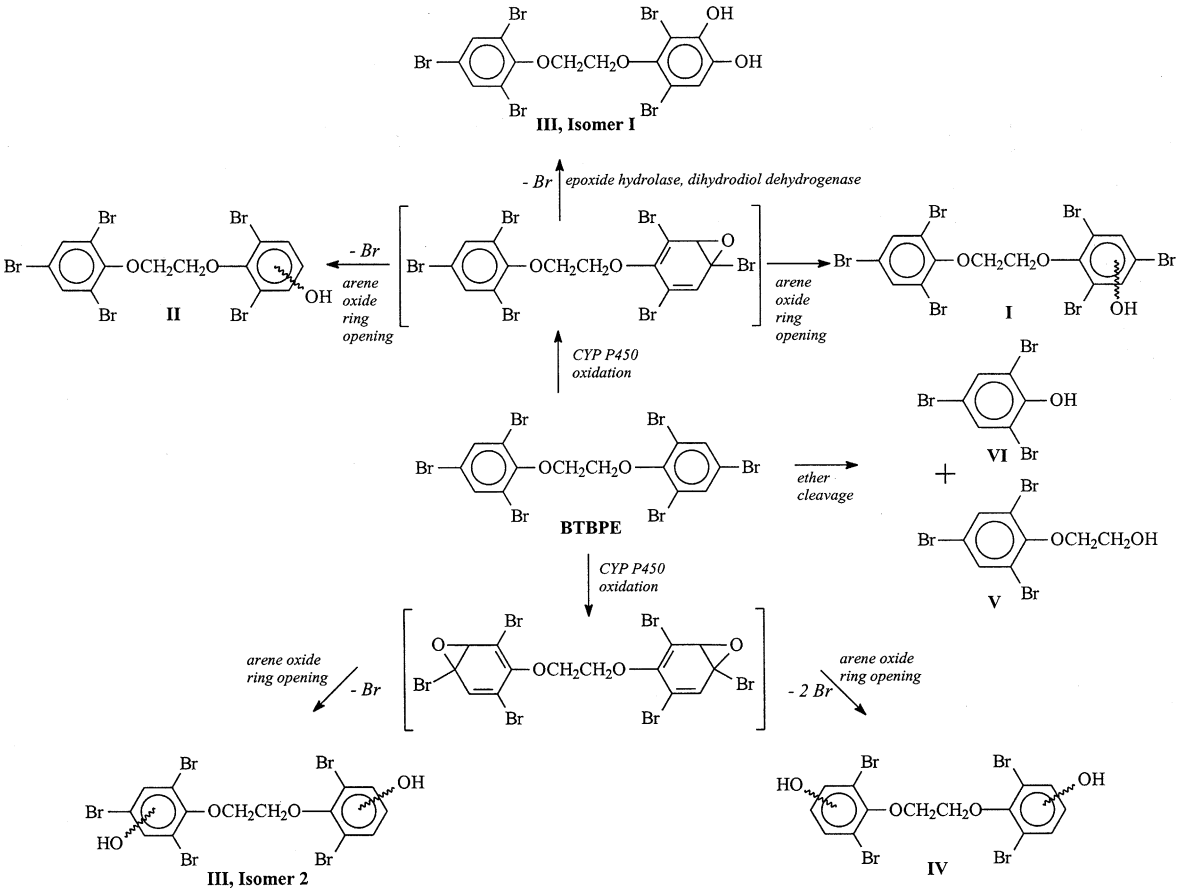



Fig. 1. Proposed pathway for the metabolism of BTBPE in male rats based on the metabolites identified by mass spectrometry in the feces. Oxidation, oxidative debromination and ether cleavage are the favored biotransformations.

centrations were stomach, intestines, adipose tissue, kidney and skin. In contrast, another hexabrominated BFR, 2,4,5,2′,4′,5′-hexabromobiphenyl, the major congener present in Firemaster BP-6, was readily absorbed (>90%) from the intestines following a single oral dose (Matthews et al., 1977). However, the half-life of 2,4,5,2′,4′,5′-hexabromobiphenyl exceeded the life of the rat, because it was not subject to appreciable metabolism.

As a result of the present observations, it was concluded that biliary and urinary excretion of BTBPE would be minimal following oral exposure, and this was accompanied by low tissue levels. Less than 4% of the 2.0 mg/kg dose was subject to oxidation, oxidative debromination and ether cleavage as determined by MS of isolated metabolites detected in the feces. Perhaps the high indoor air levels of BTBPE observed in some occupational settings (Sjödin et al., 2001) may make inhalation a more preferred route of exposure. Therefore, further metabolism studies with BTBPE may be needed to evaluate this route.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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